

Application No. 09/121,798  
Amendment Dated June 11, 2004  
Reply to Office Action of February 11, 2004

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

Claims 1 – 22 (cancelled)

Claim 23 (currently amended): A method for removing endotoxin from a plasmid DNA solution comprising:

a) filtering a solution comprising plasmid DNA through one or more filters selected from the group consisting of one or more glass fiber filters and nylon filters;

a) b) contacting a the solution comprising plasmid DNA with a trimethylamino ethyl (TMAE) anion exchange chromatography resin, the solution having a conductivity at which the plasmid DNA is bound to the resin;

b) c) washing the resin to elute endotoxin; and

c) d) eluting the plasmid DNA with a step or continuous gradient of increasing conductivity.

Claim 24 (previously presented): The method of claim 23, wherein the TMAE anion exchange chromatography resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group.

Claim 25 (previously presented): The method of claim 23, wherein the plasmid DNA solution is loaded on the resin in a solution having a conductivity of less than about 50 mS/cm.

Claim 26 (previously presented): The method of claim 25, wherein the plasmid DNA is step eluted with a series of buffers of increasing conductivity in a range of from about 50 to about 90 mS/cm.

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**Claim 27 (cancelled):**

**Claim 28 (presently amended):** The method of claim 27 23, where the plasmid DNA solution is filtered through a series of filters comprising at least one glass fiber filter and at least one nylon filter prior to contacting the plasmid DNA solution with the anion exchange chromatography resin.

**Claim 29 (previously presented):** The method of claim 23, wherein the plasmid DNA solution is a clarified lysate obtained after alkaline lysis of bacterial cells comprising the plasmid DNA and removal of precipitated proteins, chromosomal DNA and cell debris.

**Claim 30 (previously presented):** The method of claim 29, wherein the clarified lysate is further neutralized to a pH of about 7 to about 8.5.

**Claim 31 (previously presented):** The method of claim 30, wherein the clarified lysate is further neutralized with a buffer that decreases an ionic strength of the lysate for direct loading onto the anion exchange resin.

**Claim 32 (previously presented):** The method of claim 30, wherein the lysate is neutralized with a buffer that comprises Tris base.

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**Claim 33 (previously presented): A method for removal of endotoxin from a plasmid DNA solution comprising:**

- a) filtering the plasmid DNA solution through a series of filters comprising at least one glass fiber filter and at least one nylon filter;
- b) loading the filtered plasmid DNA solution onto a column comprising trimethylamino ethyl (TMAE) anion exchange resin, wherein the plasmid DNA solution is loaded onto the column in a loading buffer having a conductivity below which the plasmid DNA would elute from the resin;
- c) washing the column with a buffer having a conductivity sufficient to elute endotoxin but not plasmid DNA from the resin; and
- d) eluting the plasmid DNA with a step or continuous gradient of increasing conductivity, thereby producing a solution of anion exchange purified plasmid DNA.

**Claim 34 (previously presented): The method of claim 33, wherein the plasmid DNA solution comprises a clarified lysate obtained following alkaline lysis and precipitation using continuous flow static mixers.**

**Claim 35 (previously presented): The method of claim 34, wherein the clarified lysate is neutralized to a pH of about 7 to about 8.5 prior to anion exchange chromatography.**

**Claim 36 (previously presented): The method of claim 35, wherein the clarified lysate is neutralized with a buffer that deceases an ionic strength of the lysate for direct loading onto the anion exchange resin.**

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Claim 37 (previously presented): A pharmaceutical scale method for purifying plasmid DNA comprising:

- a) mixing a solution of bacterial cells comprising the plasmid DNA with an alkaline lysis solution by flowing through a first static mixer to obtain a lysate;
- b) contacting the lysate with a potassium acetate precipitation solution by flowing through a second static mixer, thereby forming a precipitation mixture;
- c) removing a precipitate from the precipitation mixture thereby forming a clarified lysate;
- d) filtering the clarified lysate through a series of filters comprising at least one glass filter and one nylon filter thereby forming a filtered lysate;
- e) loading the filtered lysate onto a trimethylamino ethyl (TMAE) anion ion exchange chromatography resin under conditions wherein the plasmid DNA is retained on the resin;
- f) washing the resin with a buffer that removes weakly bound impurities from the resin and eluting the plasmid DNA with a step or continuous saline gradient, thereby producing a solution of anion exchange purified plasmid DNA.

Claim 38 (previously presented): The method of claim 37, further comprising the step of RNase digestion.

Claim 39 (previously presented): The method of claim 37, further comprising the step of adjusting the pH and conductivity of either the precipitation mixture or the clarified lysate to a pH in the range of about 7 to about 8.5 and a conductivity of less than about 50mS/cm prior to the filtering step wherein the filtered lysate can be directly loaded onto the anion ion exchange chromatography resin.

Claim 40 (previously presented): The method of claim 37, wherein the trimethylamino ethyl (TMAE) anion ion exchange resin comprises a methacrylate based copolymer having a tentaclc linked TMAE functional group.

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**Claim 41 (previously presented):** The method of claim 37, further comprising the step of purifying the plasmid DNA solution using ultrafiltration in the presence of a gel layer that is allowed to form before starting ultrafiltration.

**Claim 42 (previously presented):** The method of claim 41, wherein the ultrafiltration unit is an open channel tangential flow ultrafiltration unit.

**Claim 43 (previously presented):** A method for purifying plasmid DNA comprising:

- a) lysing the bacterial cells by alkaline lysis and precipitation through continuous flow static mixers to provide a lysate;
- b) clarifying the lysate and adjusting the pH and conductivity of the lysate to a pH of about 7.0 to about 8.5 and a conductivity of less than about 50mS/cm;
- c) filtering the clarified and adjusted lysate through a filter series comprising a glass filter and a nylon filter to provide a filtered lysate;
- d) purifying the plasmid DNA by anion exchange chromatography using a methacrylate based copolymer resin having a tentacle linked TMAE functional group; and
- e) optionally, ultrafiltering and diafiltering the anion exchange purified plasmid DNA through a tangential flow open channel device in the presence of a gel-layer that is formed by an initial period of recirculation.